Predicting Natural Neuroprotection in Marine Mammals: Environmental and Biological Factors Affecting Vulnerability to Acoustically Mediated Tissue Trauma in Marine Species

Terrie M. Williams
Center for Ocean Health- Long Marine Lab
Department of Ecology and Evolutionary Biology
100 Shaffer Road
University of California-Santa Cruz
Santa Cruz, CA 95060
phone: (831) 459-5123 fax: (831) 459-3383 email: williams@biology.ucsc.edu

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LONG-TERM GOALS

The primary goal of these studies is to investigate the relative vulnerability of marine mammals to acoustically mediated trauma from emboli formation. By evaluating key environmental, behavioral and physiological factors involved in the movement of gases at the whole animal and tissue levels we intend to identify factors contributing to lipid, nitrogen, and carbon dioxide gas mobilization, and concomitant tissue damage at depth. The results of this project will enable the development of environmentally sensitive schedules for oceanic acoustic activities by identifying those species most susceptible to tissue injury.

OBJECTIVES

To accomplish these goals we are focusing on three key questions:

1. ***Environmental: Does elevated environmental temperature compromise the dive response that safeguards marine mammals from decompression illness?*** This is being tested by measuring cardiovascular and metabolic parameters of trained bottlenose dolphins during sedentary and active periods while diving in warm and cold water.

2. ***Behavioral: Do increased levels of neuroprotecting globins in the brain correspond to increased plasticity of the dive response during voluntary activity by marine mammals?*** Here we evaluate the physiological significance of elevated globin levels that we have discovered in the cerebral cortex of marine mammals. This is being tested by comparing behaviorally induced variability in the dive response (as manifested by changes in the level of bradycardia and peripheral circulation) in deep and shallow diving mammal species including bottlenose dolphins and beluga whales.

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3. ***Physiological*: *Does globin deposition and coincident neuroprotection of the cerebral cortex change with developmental stage in marine mammals?*** By evaluating globin deposition profiles from carcasses ranging in age from neonates to adults, we are investigating how age influences neuroprotective mechanisms in a wide variety of marine mammal species.

Together these studies will enable us to determine if some marine mammal species, such as the family of beaked whales, are more susceptible to non-auditory tissue damage as may occur in conjunction with navy and oil exploration sound operations. We will take into account several recent hypotheses regarding emboli formation, observed behavioral responses of marine mammals to low- and mid-frequency sound production, as well as the results of our studies to develop predictive models for susceptibility to decompression illness.

APPROACH

This study uses two approaches to determine the relative susceptibility of different marine mammal species to acoustically mediated trauma, 1) molecular and biochemical evaluation of neuroprotection at the tissue level, and 2) whole animal/physiological assessments to determine the impact of behavioral and environmental challenges to the dive response. Because stranded marine mammals often display behaviors associated with neural dysfunction (i.e. disorientation, poor localization and righting responses), and neural tissues are exceptionally vulnerable to decompression damage, we focus on the central nervous system and its relationship to the dive response.

Laboratory studies at the tissue level are assessing the presence and function of oxygen binding circulating (hemoglobin) and resident (cytoglobin and neuroglobin) globin proteins in the brain. Recently, a survey of shallow and deep diving species enabled us to determine the effects of routine dive capacity on the expression of these globins (Williams *et al.*, 2008). Our current studies build on this foundation to evaluate how these different globins affect the vulnerability of a variety of marine mammal species to hypoxia associated with decompression syndromes. Because the concentration of other globin proteins (i.e. myoglobin) changes with developmental stage in marine mammals, we are also examining how age influences globin deposition and coincident neuroprotection in the brain of immature and mature marine mammals. Ultimately, this will allow us to determine if specific segments of marine mammal populations are more susceptible than others to neural damage.

Team members include specialists in morphology and pathology of marine mammals (M. Miller, CA Dept. Fish and Game; D.A. Pabst, Univ. North Carolina-Wilmington), globin chemists (D. Kliger and R. Goldbeck, UCSC), molecular biologists (M. Zavanelli, UCSC) and physiologists (T.M. Williams and D. Casper, UCSC).

The second component of this study examines the susceptibility of marine mammals to decompression illness at the whole animal/physiological level by monitoring behaviorally induced variability in the dive response. Because nitrogen transfer and decompression illness are linked to tissue perfusion, relaxation of the dive response in marine mammals has the potential to increase susceptibility to neural tissue damage either by preventing the removal of nitrogen or altering the perfused tissue pool available for nitrogen dispersal. The effects of two physiological mechanisms known to alter blood flow are being investigated, exercise and heat. In the first series of tests we are evaluating the effects of exercise intensity on changes in the dive response of bottlenose dolphins. Dolphins are trained to dive and exercise at varying depths. Variability in bradycardia and peripheral vasoconstriction are

subsequently monitored as the animals perform sedentary to high intensity exercise tasks. Our most recent work provides a comparative dimension by conducting similar tests on a deep diving species, the beluga whale, and a free-ranging deep diver, the Weddell seal. A second set of tests uses this protocol is determining the effects of acute and chronic increases in environmental temperature on variability of cardiovascular responses in diving bottlenose dolphins.

Team members for this part of the program include physiologists (T.M. Williams, S. Noren, and L. Yeates from UCSC) and animal behaviorists (T. Kendall and B. Richter, UCSC; P. Berry, EPCOT; W. Hurley, GA Aquarium)

WORK COMPLETED

Tissue Globin Analyses. Our team has successfully developed two assays for brain globins, a spectrophotometric test that provides total globin concentration and an mRNA expression test for relative cytoglobin and neuroglobin levels. Previously, we have used these assays to detect the presence and concentration of globins in the cerebral cortex of 16 species of mammals. This includes five species of terrestrial mammal ranging in body mass from 0.1 kg to 100 kg, and 11 species of marine mammal ranging in mass from 30 to 300 kg. Among the marine species, we have examined both coastal and pelagic divers among the small cetaceans, pinnipeds and sea otters. All have demonstrated the presence of globins, although the concentration varies among the various species. We are currently refining our isolation techniques in order to quantify the level of globins as well as characterize the exact molecular structure of the globins. Furthermore, we have collected brain samples representing different ages from several marine mammal species including sea otters, pinnipeds and cetaceans. Analysis of these tissues is ongoing.

Variation in Diving Bradycardia and Vascular Control during Diving. The second component of this study examines variability in the dive response of cetaceans due to exercise and environmental temperature. A major challenge was developing heart signal instrumentation that could withstand the rapid swimming movements of dolphins. Last year we successfully tested and collected data using a new submersible electrocardiograph/accelerometer monitor by UFI (Morro Bay, CA). To date eight dolphins, two beluga whales, and two Weddell seals have been examined. Heart rate during surface and submerged resting periods were collected for all three species, including an evaluation of the effects of body position on bradycardia. During the past year we deployed our instrument on free-ranging Weddell seals to provide comparative data for a deep-diving pinniped during foraging events.

We have completed a series of exercise tests for dolphins freely-diving to 3 m, 10 m and 20 m. We have also collected data on the combined effects of exercise and increased environmental temperature on cardiovascular responses in dolphins using heat flow and changes in core body temperature as metrics for alterations in blood flow. Data analysis for the dolphins is ongoing and includes cardiovascular signatures for each level of exercise intensity and water temperature. Comparative studies on the beluga whales and Weddell seals are scheduled to continue this year. The results from the dolphin tests have been presented at the **Society for Integrative and Comparative Biology** meeting (Seattle WA, January 2010). Predictive models of gas movement in the cardiovascular system, aerobic dive limits, and susceptibility to decompression illness based on our results were discussed at the **Diving Marine Mammal Gas Kinetics Workshop** (Woods Hole MA, April 2010) and are being incorporated in three manuscripts currently in preparation with anticipated submission dates of December 2010.

RESULTS

A major accomplishment during this year was a comparative assessment of the effects of exercise on bradycardia in diving dolphins and Weddell seals. With the development of an ECG-accelerometer microprocessor we have, 1) correlated discrete changes in heart rate with propulsive stroking, 2) determined the effects of preferred, trained, and free-ranging exercise on diving bradycardia, and 3) examined the variation in interbeat interval during diving bradycardia for low and high speed swimming for both a cetacean and deep-diving pinniped. In contrast to the invariant bradycardia reported in the literature, marine mammals appear to exhibit considerable variation in submerged heart rate that is associated with the intensity of activity. Submerged activity resulted in a consistent override of bradycardia in the diving animals, progressively increasing from sedentary stationing to slowly swimming at preferred speeds to rapid maneuvers such as quick turns or ascents.

A second major accomplishment was an evaluation of the synergistic effects of water temperature and exercise on peripheral circulation in diving dolphins (Fig. 1). Exercise in water ranging from 23.2 °C to 25.4 °C resulted in an increase in peripheral circulation, as monitored by heat flow across the flukes and dorsal fin. This occurred despite a diving response that typically includes peripheral vasoconstriction. Thus, the combined effect of exercise and warm environmental temperatures resulted in a thermoregulatory response that temporarily overrode of the dive response. The increases in heat flow also corresponded to increases in core body temperature that could take over 20 minutes on the water surface to resolve following high level exercise bouts.

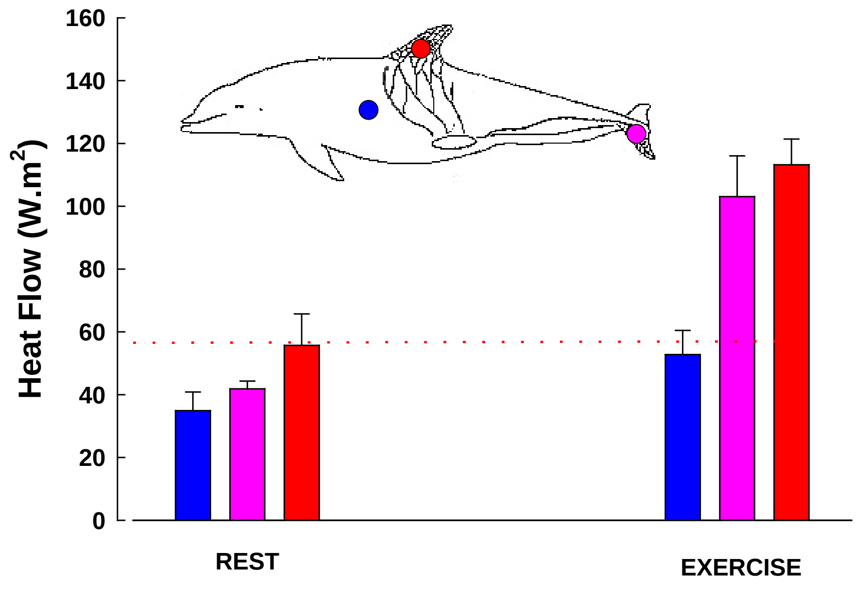

Figure 1. Variability in diving heat flow responses in the bottlenose dolphin. The colored bars denote the measured level of heat flow exhibited for the flank (blue), flukes (pink), and dorsal fin (red) by submerged dolphins during rest or following swimming exercise. The horizontal red line denotes the expected heat flow response associated with the diving response. For both peripheral sites the level of heat flow was significantly increased with activity level. Bar height and lines denote mean + 1 SE, n = 7 trials. The illustration shows the measurement sites and the vascular connection between the appendages and core organs.

Together these studies indicate a heretofore unknown level of cardiovascular variability during diving in marine mammals. Because this has marked implications for the mobilization of gases during diving we have begun constructing a predictive model to determine species specific susceptibility to decompression illness based on static and variable dive responses. Major oxygen stores, globin deposition, diving capability, and cardiovascular variability associated with exercise and environmental temperature are being incorporated into the model. These preliminary results indicate that neuroprotection in diving marine mammals may be a species-specific balance between intrinsic and extrinsic factors. A suite of oxygen-binding globins appear to provide complimentary mechanisms for facilitating oxygen transfer into neural tissues as well as the potential for protection against reactive oxygen and nitrogen groups when marine mammals are submerged. A variable cardiovascular response when submerged enables the animals to meet the demands of exercise and thermoregulation but raises a question regarding the mobilization of gases (oxygen, carbon dioxide and nitrogen) during diving.

IMPACT/APPLICATIONS

Our recent findings on variability in the cardiovascular response to diving and in tissue globin levels in the cerebral cortex provide:

1. A new perspective on neuroprotection. By examining a wide variety of mammalian species living in different habitats, we demonstrate how malleable the mammalian brain can be when placed under extreme chronic hypoxia, which occurs not only in air-breathing vertebrates who dive but also in response to various common medical conditions in humans and other species.

2. An assessment of the importance of globin proteins. Since neuroglobin and cytoglobin have been associated with neuronal survival following stroke and other ischemic insults with cardiovascular accidents, the results are relevant to many of the leading causes of mortality in the United States. Furthermore, although further research is needed, differences in resident neuroglobins may help to explain the relative susceptibility of deeper diving species to barotrauma following exposure to anthropogenic noise.

3. New techniques for clinical, ecological, behavioral and physiological studies. The instrumentation developed for monitoring cardiovascular changes in freely-diving marine mammals provides a new tool for assessing the response of wild mammals to anthropogenic disturbance. In addition, our study is developing new biochemical methods and animal models for the assessment of brain globins that should be of interest to a wide variety of comparative and medical neurophysiologists.

RELATED PROJECTS

None.

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HONORS/AWARDS/PRIZES

T.M. Williams (2010) Distinguished Ecologist Lecturer, Colorado State University, Fort Collins, CO.